Overexpression of Kinesins Mediates Docetaxel Resistance in Breast Cancer Cells

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Abstract

Resistance to chemotherapy remains a major barrier to the successful treatment of cancer. To understand mechanisms underlying docetaxel resistance in breast cancer, we used an insertional mutagenesis strategy to identify proteins whose overexpression confers resistance. A strong promoter was inserted approximately randomly into the genomes of tumor-derived breast cancer cells, using a novel lentiviral vector. We isolated a docetaxel-resistant clone in which the level of the kinesin KIFC3 was elevated. When KIFC3 or the additional kinesins KIFC1, KIF1A, or KIF5A were overexpressed in the breast cancer cell lines MDA-MB231 and MDA-MB 468, the cells became more resistant to docetaxel. The binding of kinesins to microtubules opposes the stabilizing effect of docetaxel that prevents cytokinesis and leads to apoptosis. Our finding that kinesins can mediate docetaxel resistance might lead to novel therapeutic approaches in which kinesin inhibitors are paired with taxanes. [Cancer Res 2009;69(20):8035–42]

Introduction

Systemic chemotherapy of breast cancer has improved over the past few decades with the introduction of new drugs, and the optimization of numerous combination regimens (1). An important advance has been the use of docetaxel and paclitaxel, which bind to the β-tubulin subunits of microtubules, inhibiting the rate of exchange of free and bound tubulin (2). This inhibition disrupts mitotic spindle formation, inhibiting cell division and leading to cell death (3). Although up to half of the patients treated with docetaxel achieve a clinical response, their tumors may develop resistance subsequently (3). Several different mechanisms account for the taxane resistance observed in human tumors and tumor cell lines, including overexpression of the multidrug transporter P-glycoprotein (4), altered metabolism of the taxane, decreased sensitivity to death-inducing stimuli (5), alterations in microtubule dynamics, and altered binding of the taxane to its microtubule target (6, 7). Candidate proteins that might mediate taxane resistance through their inclusion in microtubules or their association with these structures include βIII-tubulin (2), the microtubule-associated proteins MAP4 and Tau, and the microtubule-destabilizing phosphoprotein Stathmin (8, 9). Analysis of the levels of these proteins might be clinically useful in predicting responses to taxanes, but none have proven to be specific targets for increasing the efficacy of taxane-based therapy in breast cancer.

Materials and Methods

Cells and reagents. MDA MB 231 cells and MDA MB 468 cells, kindly provided by Dr. John Pink (Case Western Reserve University, Cleveland, OH), were grown in a humidified atmosphere containing 5% CO2 in RPMI 1640, supplemented with 5% fetal bovine serum (HyClone Laboratories, Inc.). Docetaxel and Vincristine were from Sigma. Anti-KIFC1 and anti-KIF5A were from Abcam, Inc. Anti–pan-actin and anti–α-tubulin were from NeoMarkers and Santa Cruz Biotechnology, respectively. Full-length cDNAs for KIFC1, KIF1A, and KIF5A were from Open Biosystems.

Plasmids. The three VBIM vectors (pVBIM SD1, SD2, and SD3), constructed on a lentiviral backbone, represent three different reading frames (10). Cre recombinase (20) was expressed from a construct in the
pBabePuro vector. To construct KIFC3 cDNA, total RNA was isolated from mutant cells by using a Qiagen kit according to the protocol provided by the manufacturer. Reverse transcription was performed using oligo DT20 primers with the SuperScript III First-Strand Synthesis System kit and protocol (Invitrogen). Standard PCR reactions were performed by using a forward primer from the VBIM vector and a KIFC3-specific reverse primer with a stop codon. The PCR product was cloned into the pCR2.1 vector (Invitrogen). The DNA was digested with EcoR1 and recloned into the retroviral vector pLPCX. KIFC1, KIF5A, and KIF1A were also recloned into the pLPCX vector.

Lentiviral and retroviral transduction. Lentiviruses were produced by transient transfection of 293T cells with the plasmids pVBIM-SD1, pVBIM-SD2, and pVBIM-SD3 along with a second-generation packaging construct (pCMV-dR8.74) and pMD2G both provided by D. Trono, University of Geneva, Geneva, Switzerland (21).

Generation of a VBIM-mutated cell library and selection of docetaxel resistant clones. MDA-MB 231 cells were plated at 10,000 cells per well in 48-well plates and infected with pVBIM SD1, SD2, and SD3 (20 pools for each), with 12 pools left uninfected. The cells were grown for 2 d and plated in six-well plates. When the cells attained 80% confluency, 300,000 cells were replated in each well of six-well plates and allowed to attach overnight. The cells were treated with 10 nmol/L docetaxel and kept under selection for 24 h.

Validation of docetaxel-resistant clones. Colonies surviving in docetaxel were expanded and infected with the retroviral vectors pBabe puro or pBabe Cre recombinase. The cells were selected with puromycin for 2 wk to generate stable pools. The Cre and vector control cells were plated at 300,000 cells per well in six-well plates and treated with docetaxel (10 or 12 nmol/L) for 24 h. After 1 week, the cells were stained with methylene blue.

Mapping the inserts and detection of overexpressed molecules. To identify insertion sites, inverse PCR was performed with EcoR1- and MfeI-digested genomic DNA from mutant clone SD1.10, which was circularized by self-ligation and subjected to nested PCR (10). The PCR product was cloned into pCR2.1, sequenced, and aligned to the human genome database. The insertion in the KIFC3 gene was confirmed by reverse transcription-PCR (RT-PCR), using the vector-derived forward primer and a KIFC3-derived reverse primer. Western analysis was performed using anti-KIFC3.

Cell survival assay. Cells (300,000) were plated in each well of six-well plates, allowed to attach overnight, and treated with docetaxel or vincristine for 24 h. After 7 to 10 d, the cells were lysed with 0.5 mol/L NaOH and diluted 50-fold before the A260 was measured, as an indication of the total amount of nucleic acid. The fraction of surviving cells was calculated relative to untreated controls.

Immunoblotting and mRNA expression. Western analyses were performed as described before (21). The membranes were probed with antibodies against KIFC3, KIF1C, and KIF5A or anti–pan-actin, followed by incubation with anti-mouse or anti-rabbit secondary antibody-horseradish peroxidase conjugates (Hoffman-La Roche). Chemiluminescence was developed by using an enhanced chemiluminescence kit (Perkin-Elmer). To

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Identification and characterization of docetaxel-resistant SD1.10 cells. A, MDA-MB 231 breast cancer cells were infected with the three VBIM insertional mutagenesis viruses and selected in docetaxel (Doce). The docetaxel-resistant mutant clone SD1.10 was infected with control vector (pBabePuro) or with a vector encoding Cre recombinase. The cells remaining after docetaxel treatment were stained with methylene blue. B, to identify the responsible gene, a PCR fragment from mutant SD1.10 was cloned, sequenced, and aligned to the human genome database. The insertion maps to the KIFC3 gene, and the VBIM integration was in the third intron of this gene, in the sense direction. C, overexpression of KIFC3 mRNA in SD 1.10 mutant cells. RT-PCR was performed with RNAs from parental cells, the SD1.10 mutant infected with control vector, and SD1.10 cells expressing Cre. Overexpression of KIFC3 mRNA was observed in the mutant at an early (22nd) PCR cycle, and this expression was completely reversed by Cre. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. D, overexpression of the KIFC3 protein in SD1.10 cells. Western analysis was performed with anti-KIFC3 antibody. Anti–pan-actin was used as a loading control. Two independent experiments gave very similar results.
determine the mRNA expression level, total cellular RNA was extracted using a Qiagen kit. The RT reaction was performed using the SuperScript III First-Strand Synthesis System, and PCR was done using KIF1A-specific primers.

**Measurement of free or polymerized tubulin.** Cells were plated in 60-mm plates (40 × 10^4 cells/plate) and allowed to attach overnight. The cells were treated with docetaxel for 24 h or left untreated. After 2 d, free or polymerized tubulin was extracted, using a standard protocol (22). Briefly, the cells were washed with PBS at 37°C, and then with 1 mL of Buffer A [containing 0.1 mol/L MES (pH 6.75), 1 mmol/L MgSO4, 2 mmol/L EGTA, 0.1 mol/L EDTA, and 4 mol/L glycerol], which prevents microtubule depolymerization. The soluble proteins were then extracted at 37°C for 5 min in a buffer (Buffer A plus 0.1% v/v Triton X-100 and protease inhibitors) and the extracts were centrifuged for 5 min at 13,000 rpm. The pellet fraction and lysed cells in the culture dish were dissolved in a buffer containing 25 mmol/L Tris (pH 6.8), plus 0.5% SDS. Total proteins from the free or polymerized tubulin fractions were analyzed by the Western method, with anti-α-tubulin. The intensity of each band was measured by using NIH software (image J) and the percentages of free and polymerized tubulin in each cell extract were quantified.

**Apoptosis assay.** Apoptosis was measured by flow cytometry, using the Annexin V FITC Apoptosis Detection kit (Calbiochem) according to the manufacturer’s protocol.

**Analysis of gene expression in breast cancers.** The “Cel” files for Affymetrix Hg 133 Plus 2 arrays used by Chang and colleagues (ref. 23; series GSE349 and GSE350) were downloaded from the National Center for Bioinformatics Information’s auxiliary Web site Gene Expression Omnibus (GEO)^4^, and the signals were analyzed by the Gene Expression and Genotyping Facility of the Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio, using Affymetrix proprietary software, Gene Chip Operating Software. All samples were scaled to 500 in accordance with the recommendations of the vendor (Affymetrix). Signal data were exported as excel spreadsheets and sorted into resistant and sensitive groups. Gene probe set identifiers and annotations for members of the family of kinesin genes were found in the Affymetrix NetAffx Analysis Center^5^ and exported as tab delimited text. These identifiers were associated with the respective signal profile records found in the Affymetrix results, using MS Access database manager. Gross profiles were then inspected to see if there were any consistent differences in expression of members of the kinesin family of genes between docetaxel-resistant and -sensitive samples.

**Statistical analysis.** Values were expressed as means ± SD. P values were based on the paired t test and the significance was set at 0.05 (marked with an asterisk wherever data are statistically significant).

**Results**

**Isolation of a docetaxel-resistant clone.** Using three varieties of VBIM lentiviral vectors, intended to allow expression in all three reading frames (10), we inserted the strong CMV promoter approximately randomly into the genomes of docetaxel-sensitive, estrogen receptor–negative MDA-MB 231 breast cancer cells. A total of 60 pools (10,000 virally infected cells per pool; 20 each with pVBIM SD1, SD2, and SD3) were selected with 10 nmol/L docetaxel. After selection, 8 of the 60 pools yielded resistant colonies; there were no colonies in 12 pools of uninfected control cells. Cells from each positive pool were infected with a lentivirus encoding Cre or with the control pBabePuro virus, treated with docetaxel and grown to test for reversion. Clone SD1.10 was validated in this way (Fig. 1A) but the other seven clones were not validated, showing that their resistance was not caused by promoter-driven events.

**Identification of KIFC3 as the gene responsible for docetaxel resistance.** A PCR fragment from mutant SD1.10 was cloned and

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^5^ [http://affymetrix.com/analysis/index.affx](http://affymetrix.com/analysis/index.affx)
sequenced. The insertion maps to the KIFC3 gene, located on human chromosome 16q13-q21. The full coding sequence of 2,061 bp of KIFC3 translates to 687 amino acids (24). The insertion in SD1.10 mapped to the third intron of the gene (Fig. 1B). Hoang and colleagues (24) showed that the translational start site is within exon 5, and therefore, the viral integration in the third intron leads to the overexpression of the complete coding sequence of KIFC3. Using a 5' VBIM-specific primer and a 3' gene-specific primer, analysis by RT-PCR showed the overexpression of the VBIM-specific KIFC3 mRNA in mutant SD1.10 and Cre recombinase eliminated this expression (Fig. 1C). The level of KIFC3 protein was significantly higher in mutant SD1.10 than in parental cells (Fig. 1D). These results indicate that the overexpression of full-length KIFC3 protein is responsible for the mutant phenotype of SD1.10 cells.

High expression of KIFC3 increases resistance to docetaxel in naïve cells. To confirm KIFC3 as a mediator of docetaxel resistance, a full-length KIFC3 cDNA was introduced into unmutagenized, MDA-MB 231 cells, resulting in stable pools of cells in which the KIFC3 protein is overexpressed (Fig. 2A). We noted two bands in western analyses of KIFC3 protein (Fig. 2A and C), perhaps due to posttranslational modifications. These cells were significantly more resistant to 4 or 6 nmol/L docetaxel than were the controls (Fig. 2B). The mixed population of cells expressing KIFC3 contains individual cells with different levels of expression, causing some cells in the population to be more resistant than others. We isolated individual clones with different levels of KIFC3 (Fig. 2C) and analyzed their resistance to docetaxel (Fig. 2D). Clones K1 and K3, which have substantially higher levels of KIFC3 protein than clone K2, were correspondingly more resistant to docetaxel. These results confirm the role of KIFC3 in docetaxel resistance in MDA-MB 231 breast cancer cells in vitro and also show that the level of KIFC3 protein correlates with the level of resistance.

Overexpression of KIFC1, KIF1A, or KIF5A increases resistance to docetaxel. In a previous study (23), core biopsy samples were taken from 24 primary breast tumors before treatment and the response of the tumors to neoadjuvant docetaxel was assessed by analysis of RNA extracted from biopsy samples. We used the Cel files for these Affymetrix Hg U95-Av2 arrays (series GSE349 and GSE350), from the National Center for Bioinformatics Information’s auxiliary Web site GEO to analyze the signals. In these data, kinesins KIFC1, KIF1A, and KIF5A were slightly overexpressed in docetaxel-resistant tumor samples, suggesting that these kinesins might have a role in mediating resistance in vivo. Therefore, we determined whether KIFC1, KIF5A, and KIF1A could mediate docetaxel resistance in our in vitro system, by generating stable MDA-MB 231 cell lines expressing them (Fig. 3). Upon exposure to 4 or 6 nmol/L docetaxel, there was a significant increase in the percentage of surviving KIFC1-expressing cells compared with control cells infected with vector alone (Fig. 3A). Increased cell survival was also observed in cells expressing KIF5A (Fig. 3B) and KIF1A (Fig. 3C). We used the same approach with another estrogen receptor-negative breast carcinoma cell line, MDA-MB 468, creating cell pools overexpressing the four kinesins (Fig. 4) and analyzing the resistance to docetaxel. Cell survival was significantly higher in KIFC3 overexpressing cells compared with vector control cells (Fig. 4A), showing that the effect of KIFC3 in docetaxel resistance is not restricted to a single cell type. Similarly, we created MDA-MB 468 cells overexpressing the other three kinesins. Upon treatment with docetaxel, cells expressing KIFC1 (Fig. 4B), KIF5A (Fig. 4C), or KIF1A (Fig. 4D) survived much better than control cells, indicating that four different kinesins can mediate docetaxel resistance in two different breast cancer cell lines.

Kinesins KIFC3, KIFC1, and KIF5A oppose the microtubule-stabilizing effect of docetaxel. Taxanes stabilize microtubules, thereby inhibiting cell processes that depend upon microtubule dynamics (25). To learn more about how KIFC3 confers resistance to docetaxel, we determined whether it affects microtubules by measuring the amounts of free tubulin in MDA-MB 231 cells overexpressing this kinesin, with or without docetaxel treatment. In response to 4 nmol/L docetaxel, the amount of free tubulin was substantially decreased in a pool of cells infected with empty vector (Fig. 5A), and at 6 nmol/L docetaxel, no free tubulin was detected.
However, in a pool of cells expressing KIFC3, the amount of free tubulin was decreased much less. Therefore, KIFC3 expression opposes the stabilization of microtubules that results from docetaxel treatment. In addition to KIFC3, we also observed a substantial increase in the level of free tubulin in MDA-MB 231 cells overexpressing KIFC1 (Fig. 5B) or KIF5A (Fig. 5C), compared with the controls, upon docetaxel treatment. However, treatment with docetaxel had no effect on free tubulin pools in cells expressing KIF1A (Fig. 5D). These results suggest that kinesins KIFC3, KIFC1, and KIF5A oppose the microtubule-stabilizing effect of docetaxel. However, KIF1A increases resistance to docetaxel by a different mechanism, which may also be used by the other kinesins (see the Discussion).

**KIFC3 inhibits docetaxel-mediated apoptosis.** Because taxane-mediated disruption of microtubules ultimately drives cells to apoptosis, we examined whether KIFC3 expression affects apoptosis in response to docetaxel in breast cancer cells. Pools of MDA-MB 231 cells overexpressing KIFC3, or control cells, were exposed to different concentrations of docetaxel for 24 hours, and after 8 days, an apoptosis assay was performed. After exposure to docetaxel, the percentage of apoptotic cells was significantly higher in control cells than in cells expressing KIFC3 (Fig. 6A).

**Microtubule depolymerization contributes to docetaxel resistance in breast cancer cells.** Free tubulin levels were substantially higher in MDA-MB 231 and MDA-MB 468 cells compared with MCF7 and T47D cells (Fig. 6B). Cell survival assays were performed to determine whether increased levels of endogenous microtubule depolymerization correlate with docetaxel resistance. Consistently, survival was greater in MDA-MB 231 and MDA MB 468 cells by 2.5- to 3-fold, compared with MCF7 and T47D cells, following treatment with 3 or 4 nmol/L docetaxel (Fig. 6C). Furthermore, when cells were exposed to the microtubule depolymerizing Vinca alkaloid Vincristine (Fig. 6D), we observed increased resistance in cells with elevated polymerized tubulin (MCF7 and T47D), compared with those with elevated free tubulin (MDA-MB 231 and MDA-MB 468). These results suggest that the relative levels of free and microtubule-bound tubulin may be useful as markers of docetaxel resistance and vincristine sensitivity in breast cancer.

**Discussion**

In cancer cells, resistance to chemotherapy is often due to the overexpression of a specific protein, often achieved through amplification of the corresponding gene. Earlier reports described several different function-based screens to discover cellular genes whose inactivation or overexpression yielded phenotypes of interest (26–28). We developed a novel validation-based insertion...
mutagenesis method to generate mutant cell lines through the overexpression of a protein due to promoter insertion (10). Here, we have used this method to discover that the overexpression of the kinesin KIFC3 confers resistance to docetaxel in breast cancer cells. Several studies show that the cellular target of taxanes is β-tubulin within microtubules (29), and taxane resistance is thought to involve alterations in microtubule structure and/or function (30). Our discovery that overexpression of the kinesin KIFC3 mediates resistance to docetaxel in breast cancer cells represents a previously unknown mechanism. Overexpression of KIFC3 increases the survival of two estrogen receptor–negative metastatic breast cancer cell lines, MDA-MB 231, and MDA-MB 468, upon treatment with docetaxel. We analyzed these particular cell lines because docetaxel is used to treat estrogen receptor–negative breast cancer patients (25, 31).

Several studies (23, 32, 33) have used microarrays to study drug-resistance mechanisms in breast cancer. Chang and colleagues (23) analyzed gene expression in core biopsy samples of primary breast cancers taken from patients resistant (>25% residual tumor volume) or sensitive (<25% residual tumor volume) to docetaxel treatment. There were two probe sets for KIFC3 gene on the affymetrix Hg U95-Av2 chip. One probe set showed a very slight increase (1.2-fold) in docetaxel-resistant compared with docetaxel-sensitive tumors and the other showed a decrease of similar magnitude (0.8-fold). We noted that the kinesins KIFC1, KIF5A, and KIF1A were slightly overexpressed in docetaxel-resistant tumors compared with docetaxel-sensitive tumors, and thus, we investigated the possible role of these three kinesins in docetaxel resistance in breast cancer cell lines, finding that overexpression of these kinesins indeed does mediate docetaxel resistance in breast cancer cell lines. KIFC1, a minus end–directed, microtubule-dependent molecular motor, is required for bipolar spindle formation. It is very similar to the kinesins ncd and kar 3, and is reported to be involved in mitosis (34). KIF1A is an anterograde motor protein that transports membranous organelles along axonal microtubules. It may play a critical role in the development of axonal neuropathies resulting from impaired axonal transport (13). A recent study showed that KIF1A is up-regulated in

![Figure 5](image_url)

**Figure 5.** Kinesins KIFC3, KIFC1, and KIF5A increase the pools of free tubulin in MDA-MB 231 cells treated with docetaxel. A, cells overexpressing KIFC3 were treated with docetaxel for 24 h. After 2 d, free and polymerized (Pol) tubulin was extracted and Western analysis was performed with anti–α-tubulin. The percentages of free and polymerized tubulin in each cell extract were quantified. All of the experiments above were repeated twice, with very similar results. B, C, and D, cells expressing KIFC1, KIF5A, or KIF1A were treated with docetaxel. Free and polymerized (Pol) tubulin was extracted and Western analysis was performed with anti–α-tubulin. The percentages of free and polymerized tubulin in each cell extract were quantified. The results shown represent two independent experiments.
endometrial cancer (35). KIF5A is expressed in nerve tissues (36) and transports vesicles and membranous organelles (37). KIF5A and KIF1A are not expressed detectably in the cell lines we have analyzed but might be expressed ectopically in specific breast cancers due, for example, to gene amplification.

Microtubules are dynamic structures with lengths that are tightly regulated. Kin1 or M-kinesins (38, 39) stimulate microtubule depolymerization in an ATP-dependent fashion (40, 41). The Kin1 kinesin HsMCAK (KNSL6) is overexpressed in human cancers (42, 43) and the hamster homologue can depolymerize paclitaxel-stabilized microtubules (44). We have found that overexpression of KIFC3, KIFC1, or KIF5A increase the pools of free tubulin in breast cancer cells treated with docetaxel, suggesting that these kinesins may antagonize the effect of docetaxel at least in part by promoting the dissociation of tubulin from microtubules. This result supports the previous deduction that the expression of endogenous microtubule depolymerizing factors might favor the development of Taxol resistance (45). Our failure to observe microtubule depolymerizing activity in cells overexpressing KIF1A is consistent with the previous finding by Ogawa and colleagues (46) that KIF1A does not have this activity. It is likely that KIF1A opposes the inhibition of tubulin-microtubule association-dissociation kinetics that is caused by docetaxel rather than affecting the equilibrium between free and microtubule-bound tubulin (2), and the other kinesins are likely to have the same effect. Kinetic measurements are needed to establish this point clearly. Inhibition of microtubule dynamics by Taxol induces persistent modification of biological processes and signaling pathways, which ultimately lead to cell death through the accumulation of signals that finally reach the threshold for the onset of apoptosis (29).

The breast cancer cell lines MDA-MB 231 and MDA-MB 468, with higher levels of free tubulin, showed increased resistance when challenged with docetaxel compared with MCF7 and T47D cells, which have lower levels. Furthermore, opposite effects were noticed when the cells were exposed to vincristine, which is a clinically used microtubule-stabilizing agent. These results are supported by the previous findings that increased levels of polymerized tubulin is associated with increased microtubule stability, decreased sensitivity to microtubule-depolymerizing drugs such as the Vinca alkaloids, and increased sensitivity to the microtubule-polymerizing drug Taxol (47). Therefore, the level of microtubule depolymerization in tumors may be a predictive factor for clinical response to taxane-based chemotherapy.

In conclusion, we have shown that overexpression of four different kinesins can mediate docetaxel resistance in breast cancer cell lines and have determined that three of them increase the fraction of soluble tubulin in cells. Resistance to docetaxel is a complex phenomenon that can be orchestrated by a number of different mechanisms. Our increased understanding of the full...
References


3. ShalliK, BROWN I, HEYS SD, Schofield AC. Alterationsof Gottesman MM. Mechanisms of cancer drug resis-


27. Pavitan Y, Bjolle A, Amler L, et al. Gene expression profiling spares early breast cancer patients from...
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